1 Introduction

There is a great interest in analytical applications of molecularly imprinted polymers (MIPs), which could serve as the molecular recognition element of drug assays, biosensors, separation media, and affinity supports for screening libraries of bioactive compounds [1–5]. MIPs allow synthesis of polymers with selectivity toward a predetermined target. The advantages of MIPs, in particular high selectivity and physiochemical stability, have propelled the development of MIPs as chromatographic stationary phases, particularly, in HPLC and CEC where they have been utilized mainly as chiral stationary phases (CSPs). There are a variety of chiral compounds separated with MIP stationary phases by HPLC and CEC including amino acid derivatives, β-blockers, peptides, and sugars [6–10].

Traditionally, the molecularly imprinted CSPs have been synthesized as a bulk polymer, ground, sieved, and slurry packed into columns. This is a time-consuming operation.

Furthermore, a large amount of the template is needed in order to synthesize the large amount of MIP that is required for packing of HPLC columns, which is problematic as the template in many cases can be both rare and expensive. In other approaches, regular molecularly imprinted microspheres have been prepared by precipitation polymerization, suspension polymerization, and multistep swelling polymerization methods [11, 12]. However, many complicated procedures and reaction conditions are required. The method of in situ polymerization was employed to prepare molecularly imprinted monolithic columns in HPLC and CEC [13, 14]. Using this technique, MIPs can be synthesized directly inside stainless steel columns or capillary columns without the tedious procedures of grinding, sieving, and column packing. Recently, Huang et al. [15] have successfully separated some enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids on the molecularly imprinted monolithic columns in HPLC.

1,1′-Bi-2-naphthol is one of the most popular analytes in chiral separation since optically active 1,1′-bi-2-naphthol and its derivatives are frequently used as chiral auxiliaries and ligands in asymmetric synthesis [16]. Sibrian-Vazquez and Spivak [17] have separated the enantiomers of 1,1′-bi-2-naphthol on the MIP by bulk polymerization with HPLC. Liu et al. [18] have prepared the (S)-(−)-1,1′-bi-2-naphthol imprinted monolithic capillary column, and the enantioseparation was obtained with CEC. In this work,
we employed one enantiomer of 1,1'-bi-2-naphthol, and 5',5',6',6',7',7',8,8'-octahydro-1,1'-bi-2-naphthol (OHBN) as the template molecules to prepare molecularly imprinted monolithic columns in HPLC. After optimization of the chromatographic conditions, these two chiral compounds were resolved completely.

2 Experimental

2.1 Materials

(\(R\))-(+)-1,1'-Bi-2-naphthol, (\(S\))-(−)-1,1'-bi-2-naphthol, (\(R\))-(+)-5,5',5',6,6',6',7,7',8,8'-octahydro-1,1'-bi-2-naphthol, and (\(S\))-(−)-5,5',6,6',6',7,7',8,8'-octahydro-1,1'-bi-2-naphthol were purchased from Sigma (St. Louis, MO, USA). 4-Vinylpyridine (4-VP) from Acros (Geel, Belgium) was distilled under vacuum. Ethylene dimethacrylate (EDMA) from Sigma was extracted with 10% aqueous sodium hydroxide and water, and dried over anhydrous magnesium sulfate. 2,2'-Azobis-isobutyronitrile (AIBN) was obtained from Shanghai Chemical Plant (Shanghai, China) and recrystallized in ethanol before use. Toluene and dodecanol were dried prior to use. Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA). All other chemicals and solvents were of analytical or HPLC grade.

2.2 Preparation of molecularly imprinted monolithic columns

The (\(S\))-(−)-1,1'-bi-2-naphthol imprinted monolithic column was directly prepared within the confines of a stainless steel column (50 x 4 mm ID). The template molecule, functional monomer, cross-linker (EDMA), and initiator (AIBN) were dissolved in porogenic solvents (1.4 mmol toluene and 6.2 mmol dodecanol) to form homogenous solution, in the compositions as indicated in Table 1. Reaction solution was sonicated for 10 min and purged with dry nitrogen for 15 min to remove oxygen. The stainless steel tube sealed at the bottom was full of the above polymerization solution, and then sealed at the top. The polymerization was carried out at 50°C in a water bath for 16 h. After that, the seals of column were replaced with fittings and connected to an HPLC pump.

Similarly, the (\(R\))-(+)-5,5',6,6',6',7,7',8,8'-octahydro-1,1'-bi-2-naphthol-imprinted monolithic column was prepared according to the procedures mentioned above.

The nonimprinted blank monolithic column (BP) without presence of the templating molecules in the reaction solution was prepared in the same way.

2.3 HPLC

The chromatographic evaluation was performed on a Shimadzu LC-10A HPLC system (Shimadzu, Kyoto, Japan) consisting of two LC-10Advp pumps and an SPD-10Avp UV-Vis detector. The data were acquired and processed with WDL-95 chromatographic workstation (National Chromatographic R & A Center, Dalian, China). The column was washed thoroughly with methanol/acetic acid (4/1, v/v) to remove the porogenic solvents and the free template molecules and monomers until a stable baseline was achieved. The HPLC was carried out at room temperature at a flow rate of 0.3 mL/min. A 5 μL sample of 1.0 mg/mL ACN was injected. The detection wavelength was set at 254 nm. Acetone was injected as the void marker. The mobile phase was pure ACN with addition of acetic acid, water, or 20 mM phosphate buffer solution (PBS).

Retention factor, \(k\), was calculated by using the equation \(k = (t_b - t_0)/t_w\), where \(t_b\) and \(t_w\) are the retention times of the analyte being investigated and the void marker, respectively. The enantioseparation factor, \(α\), is defined as the ratio of the retention factor of the lately eluted to the early eluted enantiomer, \(k_2/k_1\). The resolution is calculated from the equation \(R_s = 2(t_b - t_0)/w_1 + w_2\), where \(t_b\) and \(t_0\) are the retention times of the first and second eluted enantiomers, respectively, and \(w_1\) and \(w_2\) are the baseline peak widths of the first and second eluted enantiomers, respectively.

3 Results and discussion

3.1 Effect of composition of polymerization solution on chiral resolution

The effect of the template-monomer ratio on the selectivity has been documented. Only a proper template-monomer ratio could afford high selectivity [19]. Table 1 shows the effect of molar amounts of template molecule and functional monomer on the retention factor, enantioseparation factor, and resolution of racemate of 1,1'-bi-2-naphthol. The retention factor of (\(R\))-(+)-1,1'-bi-2-naphthol increased with an increase of the amount of template molecule used by keeping 4-VP at 1 mmol. However, the maximum enantioselectivity was provided on the MIP with (\(S\))-(−)-1,1'-bi-2-naphthol at 0.3 mmol. When the molar ratio of 4-VP and (\(S\))-(−)-1,1'-bi-2-naphthol is 3.3, the retention factor and enantioselectivity increased with an increase of their amounts. However, the mobile phase could not flow through the resultant MIP column when the amounts of 4-VP and (\(S\))-(−)-1,1'-bi-2-naphthol are 1.3 and 0.4 mmol, respectively. Thus the amounts of EDMA, 4-VP, and (\(S\))-(−)-1,1'-bi-2-naphthol in polymerization solution at 4.0, 1.0, and 0.3 mmol, respectively, are the optimum compositions for the preparation of MIPs. The batch-to-batch reproducibility for the preparation of the monolith and the durability for its repeated use were also evaluated, and good results were obtained.
3.2 Effect of mobile-phase composition on the retention and chiral resolution

As shown in Fig. 1, the chromatogram obtained with mobile phase of ACN without any additives indicated that the second eluted peak is seriously broadening, tailing, and asymmetric. A mobile phase containing polar substances was used to weaken the binding of template molecule to the MIP and consequently to release them from the imprinting cavity of the stationary phase more quickly [20]. Figure 2a shows the effect of concentration of acetic acid in the range of 0–2% v/v on the retention factor of the enantiomers of 1,1′-bi-2-naphthol. As the concentration of acetic acid increased gradually, the retention of (S)-(–)-1,1′-bi-2-naphthol and (R)-(+)–1,1′-bi-2-naphthol on the MIP decreased; however, the retention of (S)-(–)-1,1′-bi-2-naphthol decreased more remarkably. The enantioseparation factor decreased from 4.3 to 3.1 on addition of 2% v/v acetic acid. It is believed that the carboxyl group in acetic acid could interact with the pyridyl group on the MIP, in competition with the template molecule, which finally eluted out from the column. As the acetic acid increased up to 5% v/v or higher, no separation was observed.

The experiments in pure ACN containing different concentrations of water in the range of 0.1–40% v/v were also performed, and similar results were obtained as shown in Fig. 2b. The retention of (S)-(–)-1,1′-bi-2-naphthol decreased sharply when the concentration of water increased gradually, whereas that of (R)-(+)–1,1′-bi-2-naphthol decreased gently. The enantioseparation factor decreased from 4.3 to 2.8 on addition of 4% v/v water. At 10% v/v water in ACN only a shoulder peak was observed. These results suggest that the hydrogen-bonding interaction between the solutes and MIP stationary phase was suppressed by water. However, as the amount of water was increased further, the retention of both (S)-(–)-1,1′-bi-2-naphthol and (R)-(+)–1,1′-bi-2-naphthol began to increase again. When the water content in ACN was higher than 40% v/v, the retention of (S)-(–)-1,1′-bi-2-naphthol was too strong to be eluted from the column. These results can be explained by considering that at lower water percentages in ACN, water molecules act as competing ligands for the hydrogen-bonding sites of the MIP and reduce both the retention and the resolution [21]. However, since the 1,1′-bi-2-naphthol and the polymer are relatively hydrophobic, the hydrophobic interaction came to play when the percentage of water was increased, the retention began to increase and the recognition was regained.

The effect of pH value on retention and enantioseparation was also investigated by addition of ACN into 20 mM PBS with different pH value. The results (not shown in paper) indicated that the retention and enantioseparation factors did not obviously change as the pH changed from 3.0 to 7.5. These results are similar with the previous reports obtained by Sanbe and Haginaka [22], which further suggest that hydrophobic interaction between 1,1′-bi-2-naphthol and 4-VP-co-EDMA materials could play an important role in the enantioseparation of (R,S)-1,1′-bi-2-naphthol. Thus, both the intermolecular hydrogen-bonding interactions and hydrophobic interactions play a significant role in the enantioseparation.
Chiral separation of 1,1′-bi-2-naphthol and its analogue

3.3 Effects of flow rate, column temperature, and sample loading on the separation of (R,S)-1,1′-bi-2-naphthol enantiomers

Table 2 shows the retention factor, enantioseparation factor, and resolution of (R,S)-1,1′-bi-2-naphthol enantiomers at flow rates of 0.1–1.0 mL/min. With a decrease in the flow rate, the retention times increased, while the best resolution was obtained at a flow rate of 0.3 mL/min. This may be due to the slow mass transfer of the enantiomers on the MIP [23]. Figure 3 shows the chiral resolution of (R,S)-1,1′-bi-2-naphthol enantiomers at column temperature of 20, 30, 40, 50, 60, and 70 °C. With an increase in the column temperature, the retention of (S)-(–)-1,1′-bi-2-naphthol decreased more remarkably than that of (R)-(+)1,1′-bi-2-naphthol, and the enantioseparation factor increased slightly, while the resolution did not obviously change.

The effect of sample loading on the chiral resolution of (R,S)-1,1′-bi-2-naphthol enantiomers was also investigated by injecting (R,S)-1,1′-bi-2-naphthol at different concentrations onto the monolithic column. The results are listed in Table 3. With a decrease of sample loading, the enantioseparation factor gradually increases, while the highest resolution is obtained when sample loading is 2.0 µg, which is larger than the value when sample loading is 1.0 µg. The small rise in resolution is due to the sharpening of the peaks as the amount of analyte is increased, which is quickly overcome by the reduction in peak separation at higher sample loading, as the numbers of high-affinity binding sites are limited on surface of MIP [24]. As can be seen from the chromatograms, the peak of (S)-(–)-1,1′-bi-2-naphthol is broadening, tailing, and asymmetric as the sample loading is lowered. Although the

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**Table 2. Effect of flow rate on the separation of (R,S)-1,1′-bi-2-naphthol enantiomers on the (S)-(–)-1,1′-bi-2-naphthol-imprinted column**

<table>
<thead>
<tr>
<th>Flow rate, mL/min</th>
<th>k_R</th>
<th>k_S</th>
<th>a</th>
<th>R_s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.24</td>
<td>3.78</td>
<td>3.05</td>
<td>1.16</td>
</tr>
<tr>
<td>0.3</td>
<td>1.18</td>
<td>3.15</td>
<td>2.67</td>
<td>1.25</td>
</tr>
<tr>
<td>0.5</td>
<td>1.12</td>
<td>2.64</td>
<td>2.35</td>
<td>0.96</td>
</tr>
<tr>
<td>0.7</td>
<td>1.04</td>
<td>2.18</td>
<td>2.10</td>
<td>0.74</td>
</tr>
<tr>
<td>1.0</td>
<td>0.99</td>
<td>1.78</td>
<td>1.80</td>
<td>0.58</td>
</tr>
</tbody>
</table>

HPLC conditions as in Table 1.

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**Figure 2.** Effects of the content of acetic acid (a) and water (b) in ACN mobile phase on retention and enantioseparation factor of (R,S)-1,1′-bi-2-naphthol on (S)-1,1′-bi-2-naphthol-imprinted column. (A) Retention factor of (R)-(+)1,1′-bi-2-naphthol, k_R; (B) retention factor of (S)-(–)-1,1′-bi-2-naphthol, k_S; (C) enantioseparation factor, a. HPLC conditions as in Table 1.

**Figure 3.** Chromatograms for enantioseparation of (R,S)-1,1′-bi-2-naphthol at different column temperatures. HPLC conditions as in Table 1.

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phenomenon of peak broadening due to the diffusion exists in any chromatographic model, this is particularly severe for MIP columns. This poor performance could be attributed to many possible reasons. In addition to the nonspecific interaction, the heterogeneous population of different energy binding sites formed in the MIP is usually the result of a partially incomplete monomer-template association [25]. Thus, the rapid enantioseparation of 1,1'-bi-2-naphthol and OHBN on the (S)-(–)-1,1'-bi-2-naphthol-imprinted monolithic columns, respectively, was obtained as shown in Fig. 4. It can be seen that these two chiral compounds were successfully discriminated within 6 min at the flow rate of 1.0 mL/min on MIP monolithic columns, respectively. It seems that this result was not in agreement with the conclusion above that the resolution decreases with an increase of the flow rate. Since the sample loading was lower, the similar resolution could be obtained. It is impossible that such a rapid chiral separation process could be performed on the conventional column packed with MIPs at high flow rate [26].

3.4 Cross-reactivity of these two MIP monolithic columns

The ability of the (R)-(+) -OHBN-imprinted monolithic column to separate the (R,S)-OHBN and (R,S)-1,1'-bi-2-naphthol is exhibited in Fig. 5a. As can be seen, the enantiomers of (R,S)-OHBN could be completely separated, but the enantiomers of (R,S)-1,1'-bi-2-naphthol could only be separated partly. When the amount of (R,S)-1,1'-bi-2-naphthol injected to the HPLC column was same to that of (R,S)-OHBN, the peaks of the enantiomers of 1,1'-bi-2-naphthol were overlapped. In addition, the chromatogram of enantioseparation of the (R,S)-OHBN on the (S)-(–)-1,1'-bi-2-naphthol-imprinted polymer was also evaluated. When the amount of (R,S)-OHBN injected to the HPLC column was similar to that of 1,1'-bi-2-naphthol, the peaks of the enantiomers of OHBN were partly overlapped. As exhibited in Fig. 5b, it can be seen that the (R,S)-OHBN enantiomers could be successfully discriminated on the (S)-(–)-1,1'-bi-2-naphthol-MIP monolithic column when the sample loading was decreased, and the values of enantioseparation factor and resolution are 3.58 and 1.02, respectively. The results show that these two chiral compounds could be enantioseparated on the same MIP column, which indicates that MIP could recognize the targets with affinity and selectivity in the same order as antibodies generated for the same target structure.

4 Concluding remarks

The molecularly imprinted monolithic columns have been prepared in stainless steel column by in situ polymerization method for chiral separation of the enantiomers of 1,1'-bi-2-naphthol. The monoliths with good flow-through properties integrate the high enantioselectivity of MIP and

Table 3. Effects of sample loading on the chiral separation of (R,S)-1,1'-bi-2-naphthol on the (S)-(–)-1,1'-bi-2-naphthol-imprinted column

<table>
<thead>
<tr>
<th>Sample, µg</th>
<th>kR</th>
<th>kS</th>
<th>α</th>
<th>Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.97</td>
<td>5.79</td>
<td>5.96</td>
<td>1.42</td>
</tr>
<tr>
<td>2.0</td>
<td>0.89</td>
<td>4.09</td>
<td>4.57</td>
<td>1.61</td>
</tr>
<tr>
<td>4.0</td>
<td>0.88</td>
<td>3.01</td>
<td>3.74</td>
<td>1.35</td>
</tr>
<tr>
<td>8.0</td>
<td>0.87</td>
<td>2.88</td>
<td>3.31</td>
<td>0.88</td>
</tr>
<tr>
<td>12.0</td>
<td>0.83</td>
<td>2.57</td>
<td>3.08</td>
<td>0.73</td>
</tr>
</tbody>
</table>
pared. These two columns have similar properties by showing a good cross-reactivity. By the combination of stepwise gradient elution and high flow rate, the baseline separation of these chiral compounds was successfully obtained within 6 min. It further shows the MIP monolith could be applied for fast chiral separation.

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5 References